



**EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA); Scientific Opinion on the substantiation of health claims related to isoleucine-proline-proline (IPP) and valine-proline-proline (VPP) and maintenance of normal blood pressure (ID 661, 1831, 1832, 2891, further assessment) pursuant to Article 13(1) of Regulation (EC) No 1924/2006 (further reassessment)**

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## SCIENTIFIC OPINION

### **Scientific Opinion on the substantiation of health claims related to isoleucine-proline-proline (IPP) and valine-proline-proline (VPP) and maintenance of normal blood pressure (ID 661, 1831, 1832, 2891, further assessment) pursuant to Article 13(1) of Regulation (EC) No 1924/2006<sup>1</sup>**

**EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA)<sup>2,3</sup>**

European Food Safety Authority (EFSA), Parma, Italy

#### ABSTRACT

Following a request from the European Commission, pursuant to Article 13.1 of Regulation (EC) No 1924/2006, the Panel on Dietetic Products, Nutrition and Allergies was asked to provide a scientific opinion on a health claim related to isoleucine-proline-proline (IPP) and valine-proline-proline (VPP) and maintenance of normal blood pressure. The food constituent that is the subject of the claim, the tripeptides isoleucine-proline-proline (IPP) and valine-proline-proline (VPP), is sufficiently characterised. The claimed effect, maintenance of normal blood pressure, is a beneficial physiological effect. The proposed target population is the general population. In weighing the evidence, the Panel took into account that 15 of the human intervention studies provided, of which seven were adequately powered to detect small between-group differences in systolic blood pressure, did not observe an effect of IPP and VPP on systolic blood pressure or diastolic blood pressure; that interpretation of the results from nine out of the ten studies which reported an effect of IPP and VPP on office systolic blood pressure was limited by methodological weaknesses; that the animal and *in vitro/ex vivo* studies did not provide additional information on the effect of IPP and VPP on blood pressure in humans; and that there is no convincing evidence for a mechanism by which IPP and VPP could exert the claimed effect in humans at the proposed dose. On the basis of the data presented, the Panel concludes that a cause and effect relationship has not been established between the consumption of IPP and VPP and maintenance of normal blood pressure.

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#### KEY WORDS

Isoleucine-proline-proline, IPP, valine-proline-proline, VPP, blood pressure, health claims.

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## SUMMARY

Following a request from the European Commission, the Panel on Dietetic Products, Nutrition and Allergies was asked to provide a scientific opinion on a list of health claims pursuant to Article 13 of Regulation (EC) No 1924/2006. The Commission has agreed with EU Member States that a certain number of Article 13 health claims would be eligible for further assessment by EFSA in order to be able to take a final decision on whether or not to include these claims in the list of permitted health claims. This opinion addresses the scientific substantiation of health claims in relation to the tripeptides isoleucine-proline-proline (IPP) and valine-proline-proline (VPP) and maintenance of normal blood pressure. The scientific substantiation is based on the information provided by the Member States in the consolidated list of Article 13 health claims, references that EFSA has received from Member States or directly from stakeholders and the additional information provided by the competent Authority of Germany for further assessment of this claim.

The food constituent that is the subject of the health claim is the tripeptides isoleucine-proline-proline (IPP) and valine-proline-proline (VPP). The Panel considers that isoleucine-proline-proline (IPP) and valine-proline-proline (VPP) are sufficiently characterised.

The claimed effect, which is eligible for further assessment, is maintenance of normal blood pressure. The proposed target population is the general population. The Panel considers that maintenance of normal blood pressure is a beneficial physiological effect.

In its earlier opinion, the Panel had considered one meta-analysis, which included nine human intervention studies, as well as six additional human intervention studies for the scientific substantiation of the claim. In the framework of further assessment, two additional meta-analyses and six randomised controlled trials were provided as pertinent to the claim. This evaluation combines the scientific references provided in the previous submission and the additional references subsequently submitted for the scientific substantiation of a claim on IPP and VPP and maintenance of normal blood pressure.

The Panel considers that the meta-analyses submitted do not provide information in addition to the individual studies considered pertinent to the claim and considered individually for the scientific substantiation of the claim.

From the 25 human intervention studies, 10 studies, nine of which had methodological weaknesses, which limit their interpretation, reported an effect of IPP and VPP on office systolic blood pressure, and four of these studies also reported an effect of IPP and VPP on office diastolic blood pressure. Fifteen human intervention studies of which seven were designed to detect small (3-9 mm Hg) differences in systolic blood pressure, did not observe an effect of IPP and VPP on systolic or diastolic blood pressure.

With respect to possible mechanisms by which IPP and VPP could exert an effect on blood pressure it was suggested in the information provided that IPP and VPP have the potential to inhibit the angiotensin converting enzyme, that they could induce the production of nitric oxide and that they may have an effect on the sympathetic nervous system. The Panel notes that no convincing data on a possible mechanism by which IPP and VPP could exert an effect on blood pressure in humans at the proposed dose levels were provided.

In weighing the evidence, the Panel took into account that 15 of the human intervention studies provided, of which seven were adequately powered to detect small between-group differences in systolic blood pressure, did not observe an effect of IPP and VPP on systolic blood pressure or diastolic blood pressure; that interpretation of the results from nine out of the ten studies which reported an effect of IPP and VPP on office systolic blood pressure was limited by methodological weaknesses; that the animal and *in vitro/ex vivo* studies did not provide additional information on the effect of IPP and VPP on blood pressure in humans; and that there is no convincing evidence for a mechanism by which IPP and VPP could exert the claimed effect in humans at the proposed dose.

On the basis of the data presented, the Panel concludes that a cause and effect relationship has not been established between the consumption of IPP and VPP and maintenance of normal blood pressure.

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**BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION**

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**TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION**

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**EFSA DISCLAIMER**

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## INTRODUCTION

The Commission has agreed with EU Member States that a certain number of Article 13 health claims would be eligible for further assessment by EFSA in order to be able to take a final decision on whether or not to include these claims in the list of permitted health claims. These claims include already assessed claims related to micro-organisms which the Panel considered to be insufficiently characterised and claims for which the NDA Panel concluded that there was insufficient evidence to establish a cause and effect relationship between the consumption of the food and the claimed effect.

Following an opinion of the NDA Panel on a health claim pursuant to Article 13 of Regulation (EC) No 1924/2006<sup>4</sup> in which the Panel concluded that the evidence provided was insufficient to establish a cause and effect relationship between the consumption of isoleucine-proline-proline (IPP) and valine-proline-proline (VPP) and maintenance of normal blood pressure (EFSA Panel on Dietetic Products Nutrition and Allergies (NDA), 2009), EFSA received additional information from the competent Authority of Germany for further assessment of this claim.

A claim on isoleucine-proline-proline (IPP) and valine-proline-proline (VPP) and maintenance of normal blood pressure pursuant to Article 13.5 of Regulation (EC) No 1924/2006 has also already been evaluated by the Panel, with an unfavourable outcome (EFSA Panel on Dietetic Products Nutrition and Allergies (NDA), 2011).

## ASSESSMENT

### 1. Characterisation of the food/constituent (ID 661, 1831, 1832, 2891)

The food constituent that is the subject of the health claim is the tripeptides isoleucine-proline-proline (IPP) and valine-proline-proline (VPP), which can be obtained through fermentation of milk by certain lactic acid bacteria, by enzymatic hydrolysis of casein, or by chemical synthesis. All clinical studies presented have been conducted with either directly fermented milk, with powdered fermented milk, or with tripeptides obtained from enzymatically hydrolysed casein. IPP and VPP can be measured in foods by established methods.

The Panel considers that the food constituent, IPP and VPP, which is the subject of the health claim, is sufficiently characterised.

### 2. Relevance of the claimed effect to human health (ID 661, 1831, 1832, 2891)

The claimed effect, which is eligible for further assessment, is maintenance of normal blood pressure. The proposed target population is the general population.

Blood pressure (BP) is the pressure (force per area unit) exerted by circulating blood on the walls of blood vessels. Elevated BP, by convention above 140 mm Hg (systolic) and/or 90 mm Hg (diastolic), may compromise the normal function of the arteries.

The Panel considers that maintenance of normal blood pressure is a beneficial physiological effect.

### 3. Scientific substantiation of the claimed effect (ID 661, 1831, 1832, 2891)

In its earlier opinion (EFSA Panel on Dietetic Products Nutrition and Allergies (NDA), 2009), the Panel had considered one meta-analysis (Xu et al., 2008), which included nine human intervention studies, as well as six additional randomised controlled trials (RCTs) (De Leeuw et al., 2009; Engberink et al., 2008; Hirota et al., 2007; Van der Zander et al., 2008a; Van der Zander et al., 2008b; Van Mierlo et al., 2009) for the scientific substantiation of the claim. The Panel noted in this assessment that although some small studies had observed a significant decrease in systolic blood pressure (SBP) with the administration of lactotripeptides at doses around 5 mg per day in untreated

<sup>4</sup> Regulation (EC) No 1924/2006 of the European Parliament and of the Council of 20 December 2006 on nutrition and health claims made on foods. OJ L 404, 30.12.2006, p. 9–25.

pre-hypertensive or moderately hypertensive subjects, these results have not been supported by large intervention trials providing daily doses of lactotripeptides in the range of 2.7 mg to 14 mg in either normotensive or untreated hypertensive subjects, and concluded that the evidence was insufficient to establish a cause and effect relationship between the consumption of IPP and VPP and maintenance of normal blood pressure.

In the framework of further assessment, two additional meta-analyses (Aubin, 2011, unpublished; Cicero et al., 2011) and six RCTs (Cicero et al., 2010; Ishida et al., 2007; Nakamura et al., 2011; Turpeinen et al., 2009; Usinger et al., 2010a; Yoshizawa et al., 2010) were provided as pertinent to the claim.

This evaluation combines the scientific references provided in the previous submission and the additional references subsequently submitted for the scientific substantiation of a claim on IPP and VPP and maintenance of normal blood pressure.

One unpublished meta-analysis (Aubin, 2011) of RCTs performed in European subjects, and two published meta-analyses (Cicero et al., 2011; Xu et al., 2008) on the effects of IPP and VPP on BP in humans, included 12, 18 and 9 studies, respectively. The Panel notes that major differences in the quality of the studies selected for the meta-analyses were not taken into account in data analysis, and that no convincing rationale has been provided which would justify the analysis of data obtained in European subjects separately from other data. The Panel considers that these meta-analyses do not provide information in addition to the individual studies considered pertinent to the claim by the applicant and described below for the scientific substantiation of the claim.

Six parallel, placebo-controlled intervention studies compared the effects of sour milk, liquid yoghurt or tablets containing IPP and VPP at doses of 3.74 to 4.26 mg/day to those of a placebo (artificially acidified milk or tablets assumed to be devoid of IPP and VPP) on BP in hypertensive subjects not on BP-lowering medications. The interventions lasted 8-12 weeks, were preceded by two to four-week run-in periods, and were followed by two to four-week post-intervention follow-ups. Office systolic BP and diastolic BP (DBP) were measured at baseline and bi-weekly during the interventions as well as during and/or at the end of the follow-up periods. The number of subjects enrolled was 36 in two studies (six drop-outs (Kajimoto et al., 2001a); four drop-outs (Hirata et al., 2002)), 70 (six drop-outs (Kajimoto et al., 2002)), 92 (11 drop-outs (Kajimoto et al., 2001b)), 107 (one drop-out (Nakamura et al., 2004)) and 122 (11 drop-outs (Ishida et al., 2007)), respectively. All statistical analyses were conducted on the results obtained in completers only. Analysis of variance (ANOVA) was used to assess treatment effects except in the study by Nakamura et al. (2004), where repeated-measures (RM-)ANOVA was used. Between-group comparisons were primarily performed using multiple pairwise comparisons (unpaired t-tests) at different time points in order to test the treatment effect. It was reported that compared to placebo, the treatment resulted in a reduction in SBP ranging from -4 to -13 mm Hg. DBP did not change (three studies) or decreased by -4 to -8 mm Hg. The Panel notes that no information is provided in the publications about the method used to allocate treatments to the enrolled subjects, and that randomisation is not mentioned in most reports. The Panel also notes that in five out of the six studies, the use of ANOVA did not properly take into account repeated measures, that statistical analyses were conducted in completers only, and that in all these studies multiple comparisons were carried out between groups without appropriate correction for multiple testing. The Panel notes that these studies report an effect of IPP and VPP on office SBP, and that in three of these studies (2001a; Kajimoto et al., 2001b; 2002) an effect of IPP and VPP on office DBP was also reported. However, the Panel considers that these six studies have methodological weaknesses, which limit the interpretation of the results.

Nakamura et al. (2011) performed another randomised, double-blind, placebo-controlled parallel study which enrolled 70 hypertensive subjects without treatment for hypertension. After an eight-week screening period, subjects were randomised to receive tablets containing 3.4 mg per day IPP and VPP or placebo for eight weeks. Office BP was measured at baseline and at the end of the eight-week intervention period by using three different devices. Between-group differences in changes of BP from baseline were assessed using two-tailed unpaired t-tests. BP results obtained from only two of the



three devices used were provided. A reduction in SBP in the lactotripeptide group as compared to placebo was reported. The reduction in DBP reached the pre-set level of significance with only one of the two devices. The Panel notes that three subjects undergoing treatment for hypertension were enrolled, that BP measurements obtained from one device were not reported, that multiple pair-wise comparisons were performed without appropriate correction, and that the effect on DBP was not consistently observed between the BP-measuring devices used. The Panel notes that this study reports an effect of IPP and VPP on SBP, and no consistent effect on DBP. However, the Panel considers that this study has methodological weaknesses, which limit the interpretation of the results.

In a randomised, single-blind, placebo-controlled, parallel study (Mizuno et al., 2005), 131 subjects with high-normal BP or mild hypertension, not on anti-hypertensive medications, were divided into four groups to consume enzymatic casein hydrolysates in tablets providing 0, 1.8, 2.5 or 3.6 mg/day of IPP and VPP (32 subjects in the control group and 33 subjects per intervention group) for six weeks, after a two-week run-in period. Each study group included 20-21 subjects with mild hypertension and 12 subjects with high-normal BP. Office BP was measured during the run-in phase, at baseline and at 1, 3 and 6 weeks of the intervention by a trained nurse. No drop-outs were reported in the study and analyses were conducted in the intention-to-treat (ITT) population. The effect of the intervention on BP was analysed by ANOVA. It is not reported in the publication how differences between groups were tested at different time points. A statistically significant difference in SBP was observed in the 3.6 mg/day dose group compared to control (exact figure not provided;  $p < 0.001$ ). No significant differences were observed for SBP between the intervention and control groups at doses of 1.8 and 2.5 mg/day, or for DBP at any dose. Changes in BP during the study were also analysed separately for subjects with high-normal and mildly elevated BP. The Panel notes that *post hoc* analyses according to basal BP have not been justified in the publication (i.e. no *a priori* stratification according to BP has been reported and interaction between treatment and baseline BP on the outcome measure has not been tested). The Panel also notes that no details were provided regarding the method used to allocate treatments to subjects, that nurses and investigators were not blinded to treatments, and that repeated measures were not properly taken into account. The Panel notes that this study reports an effect of IPP and VPP on office SBP at doses of 3.6 mg/day, and no effect on DBP. However, the Panel considers that this study has methodological weaknesses which limit the interpretation of the results.

In a randomised, double-blind, placebo-controlled, parallel, pilot study (Seppo et al., 2002), 17 mildly hypertensive subjects not on pharmacological treatment for hypertension consumed either sour milk containing IPP and VPP (5.25 mg/day) or a control milk not containing IPP and VPP for eight weeks, after a run-in period of four weeks and followed by a four-week follow-up at the end of the intervention. All subjects completed the study. Power calculations were not performed. Office BP was measured weekly during the study by a nurse who was blinded to the intervention. Changes in BP were calculated in absolute values and as percent changes from baseline within groups at every visit. Between-group differences were tested at different time-points using t-test for independent samples. At the end of the eight-week intervention period, the treatment differences for SBP and for DBP were statistically significant. However, the Panel notes that repeated measures were not taken into account in the analysis, and that adjustments for multiple comparisons were not performed. The Panel also notes the small sample size of the study. The Panel notes that this study reports an effect of IPP and VPP on office SBP and DBP. However, the Panel considers that this study has methodological weaknesses which limit the interpretation of the results.

In a randomised, double-blind, placebo-controlled, parallel study (Sano et al., 2005), 150 men and women not on anti-hypertensive medications were randomised to consume a concentrated beverage mixture of vegetable and fruit juices containing an enzymatic casein hydrolysate with IPP and VPP (3.07 mg/day) or the same mixture without IPP and VPP for 12 weeks. Office BP was measured at baseline and every two weeks during the study. A sample size of 48 subjects (24 per group) was calculated to detect a 5 mm Hg difference in SBP between groups with  $\beta = 0.20$  and  $\alpha = 0.05$ . A total of 144 subjects completed the study and entered data analysis ( $n = 72$  per group). The effect of treatment on BP was analysed using RM-ANOVA. A statistically significant decrease was observed in the intervention group compared to placebo ( $p < 0.001$ ) for SBP, but not for DBP. The Panel notes that the

mean difference between the *verum* and the placebo groups with respect to SBP at all time points was below the 5 mm Hg difference in SBP pre-defined for power calculations. The Panel also notes that in this study, investigators over-recruited the calculated sample size by three times, and that reasons for over-recruitment have not been reported in the publication. The Panel considers that this study shows a statistically significant effect of IPP and VPP on office SBP, and no effect on DBP.

The Panel notes that ten RCTs (Hirata et al., 2002; Ishida et al., 2007; 2001a; Kajimoto et al., 2001b; 2002; Mizuno et al., 2005; Nakamura et al., 2011; 2004; Sano et al., 2005; Seppo et al., 2002) reported an effect of IPP and VPP on office SBP, and that four of these studies also reported an effect of IPP and VPP on office DBP (2001a; Kajimoto et al., 2001b; 2002; Seppo et al., 2002). However, the Panel considers that nine of the RCTs have methodological weaknesses which limit the interpretation of the results.

In a randomised, double-blind, placebo-controlled, parallel study (Mizushima et al., 2004), 46 mildly hypertensive males not on anti-hypertensive medications consumed sour milk containing IPP and VPP (3.15 mg/day) or an artificially acidified control milk (devoid of IPP and VPP) for four weeks. Office BP was measured at baseline and at weeks two and four. A sample size of 62 subjects (31 per group) was calculated to detect a 5 mm Hg difference in SBP between groups with a power of 80 % and  $\alpha=0.05$ . A total of 42 subjects (22 in the intervention group and 20 in the control group) completed the study. Changes in BP between groups during the four-week intervention were not statistically significantly different using RM-ANOVA. The Panel considers that this study does not show an effect of IPP and VPP on SBP or DBP. However, the Panel notes that this study may have been underpowered with respect to BP outcomes.

Itakura et al. (2001) enrolled 44 subjects not on anti-hypertensive medications (18 hypertensive subjects and 26 subjects with normal blood pressure) into a randomised, double-blind, placebo-controlled parallel study. Subjects received either a fermented milk providing 2.6 mg/day IPP and VPP or acidified milk as a placebo for eight weeks. Office BP was measured at baseline and every other week during the intervention period, and four weeks after its cessation. Comparisons between groups were carried out using unpaired t-tests. There was no significant difference in SBP or DBP between groups. The Panel considers that this study does not show an effect of IPP and VPP on SBP or DBP. However, the Panel notes that this study may have been underpowered with respect to BP outcomes.

Yoshizawa et al. (2010) included 43 healthy post-menopausal women into a placebo-controlled parallel study. Women were randomised to one of four treatment groups: capsules containing 6.7 mg/day IPP and VPP (n=12), the same treatment plus aerobic exercise (n=11), placebo (n=10) or placebo plus aerobic exercise (n=10). Blood pressure was measured at baseline and at the end of the eight-week intervention. RM-ANOVA was used to assess the effect of interventions on BP. There was no significant difference in SBP or DBP between groups. The Panel considers that this study does not show an effect of IPP and VPP on SBP or DBP. However, the Panel notes that this study may have been underpowered with respect to BP outcomes.

Cicero et al. (2010) included 55 untreated subjects with normal or high BP into a randomised, double-blind, cross-over study. After a four-week run-in period, subjects received for four weeks either 6 mg/day IPP and VPP in a fruit juice or the same drink devoid of lactotripeptides as a placebo. At the end of the first intervention period, subjects underwent a four-week wash-out period and were then assigned the alternative intervention for four weeks. BP was measured at baseline and at the end of each intervention period. Both office and 24 h ambulatory BP were measured. ANOVA was used to assess the effects of treatments. Five patients were not included in the analysis because of non compliance with the study protocol. Overall, there was no significant difference in office SBP or DBP. Similarly, there was no significant difference in 24 h ambulatory BP. The Panel considers that this study does not show an effect of VPP and IPP on SBP or DBP.

Aihara et al. (2005) included 80 hypertensive subjects (40 with normal-high BP and 40 with mild hypertension) into a randomised, double-blind, placebo-controlled parallel study. After a one-week run-in period, subjects received tablets containing 13 mg/day IPP and VPP or a placebo for four

weeks, and were followed up for one week after the end of the intervention. Office BP was measured at baseline and weekly for five weeks. Power calculation indicated that a sample size of 20 subjects per group was sufficient to detect a 9 mm Hg difference in SBP between groups with  $\alpha=0.05$  and an 80 % power. SBP or DBP were not significantly different between the two groups during the intervention period using RM-ANOVA. The Panel considers that this study powered to detect differences of 9 mm Hg in SBP does not show an effect of VPP and IPP on office SBP or DBP.

Seppo et al. (2003) reported on a double-blind, placebo-controlled, parallel study in which 42 hypertensive subjects were randomised to consume a fermented milk containing IPP and VPP (5.25 mg/day) or a control milk (heat treated fermented milk without IPP and VPP) designed to mimic the sensory attributes of the test product for 21 weeks after a run-in period of two weeks. Nine subjects in the test group and seven subjects in the control group were taking BP-lowering medications. Home BP was measured at baseline and weekly on the same day using an automatic BP recorder. Thirty-six subjects completed the study. RM-ANOVA was used to assess the effect of the intervention on BP. Areas under the curve (AUC) for BP values were also compared between groups using t tests for independent samples. The Panel notes that no information is provided in the publication about the treatment of missing data, and that the possible confounding effects of antihypertensive medications were not taken into account. ITT analysis did not indicate any significant difference in either SBP or DBP between groups. A significant decrease in SBP (-6.7 mm Hg), but not in DBP, was observed in the intervention group compared to placebo in the per protocol (PP) analysis ( $p=0.03$ ). AUC for BP values did not significantly differ between groups. The Panel notes that the results are inconsistent and considers that this study does not show an effect of IPP and VPP on SBP or DBP.

In a randomised, double-blind, placebo-controlled, parallel study (Turpeinen et al., 2009), 62 hypertensive and hypercholesterolaemic subjects not on antihypertensive or cholesterol-lowering medications consumed 20 g/day of a vegetable oil fat spread providing 4.2 mg/day IPP and VPP and 2 g/day plant sterols or the same amount of a placebo fat spread for 10 weeks after a run-in period of four weeks. A total of 58 subjects completed the study. BP was measured twice a week at home by the study subjects using an automated sphygmomanometer. At baseline and at week 10 of the study, office BP was measured using the same technique prior to calculating central SBP (cSBP) using pulse-wave analysis in order to evaluate aortic stiffness. Power calculations and the primary outcome of the study were not reported. Analyses were performed in the ITT population with the last observation carried forward. Analysis of covariance (ANCOVA) with baseline values as covariate was applied to assess BP changes between groups. Home BP values at baseline and at the end of the intervention period were used. There was a significant decrease in home SBP in the intervention group as compared to placebo (-6 mm Hg), whereas no significant differences between groups were observed for DBP. However, the Panel notes that the statistical analysis did not appropriately take into account repeated measures of home BP, and that intermediate home BP measurements were not considered in the analysis. No significant differences between groups were observed for central SBP. The Panel notes that results for office BP measurements were not reported. The Panel considers that this study does not show an effect of IPP and VPP on SBP or DBP.

In a randomised, double-blind, placebo-controlled, parallel study (Tuomilehto et al., 2004), 60 hypertensive volunteers not on anti-hypertensive medications consumed either sour milk containing IPP and VPP (4.8 to 5.4 mg/day) or a control milk not containing IPP and VPP (i.e. traditionally fermented milk) for 8-10 weeks (phase I). An “interim analysis” revealed that SBP and DBP decreased in all study subjects regardless of the study group to which they had been assigned. After a three to four-week wash-out period, it was proposed to the subjects ( $n=58$ ) who had completed this first study to carry on for another five to seven weeks while inverting treatments (placebo in place of *verum* and *vice versa*). Out of the 40 subjects who agreed to participate in this second phase of the study (phase II), 39 completed it. The Panel notes that only phase I of this study complies with basic requirements for subjects’ enrolment and treatment allocation, and that the combination of phases I and II cannot be considered as an appropriate cross-over. Office BP was measured at weeks 3, 6, 8, 9 and 10 (phase I). RM-ANOVA was used to assess the effect of the intervention on BP. There was no significant effect

of treatment on SBP or DBP. The Panel considers that this study does not show an effect of IPP and VPP on office SBP or DBP.

In a randomised, double-blind, placebo-controlled, cross-over trial (Van der Zander et al., 2008b), 42 participants consumed a fermented milk to which IPP and VPP (8.7 mg/day) were added and a placebo milk drink (no VPP or IPP added) for four weeks each, with a four-week wash-out period in between. Office BP was measured at the beginning and end of each period. Sample size was calculated with changes in SBP as the primary outcome (42 subjects were needed to observe a 5 mm Hg difference in SBP with an 80 % power and  $\alpha=0.05$ ). ANOVA including treatment, intervention period, treatment order and subject was performed to assess the effect of the intervention on BP. The Panel notes that repeated measures were not appropriately taken into account in the data analysis. There was no significant treatment effect on either SBP or DBP. A *post hoc* sub-group analysis was conducted on the basis of baseline SBP (SBP>130 mm Hg and SBP≤130 mm Hg). The Panel notes that no interaction analysis between SBP values at baseline and SBP changes during the intervention was performed to justify *post-hoc* analyses, and that the number of subjects with SBP>130 mm Hg (and with SBP≤130 mm Hg) has not been reported. The Panel considers that this study powered to detect differences of 5 mm Hg in SBP does not show an effect of IPP and VPP on office SBP or DBP.

In a multicentre, randomised, double-blind, placebo-controlled, parallel trial (Van der Zander et al., 2008a), 275 hypertensive subjects not on anti-hypertensive medications consumed a yoghurt drink enriched with casein hydrolysate and providing IPP and VPP (10.2 mg/day) or a placebo yoghurt drink for eight weeks. Sample size was calculated with changes in office SBP as the primary outcome (135 subjects per group were needed to observe a 3 mm Hg difference in SBP with a 90 % power and  $\alpha=0.05$ ). A total of 136 subjects were randomised to the intervention group and 139 to the control group. Office BP was measured for two to three hours on two to three consecutive days at baseline and at four and eight weeks of the intervention. ANCOVA including treatment, subject and baseline BP was used on an ITT and PP (in compliant subjects) basis to assess the effect of the intervention on BP. All BP measurements were considered in the analysis. Compliance was 98 % in both groups. A total of 134 subjects in the intervention and 137 in the control group completed the study. No significant differences in SBP or DBP were observed between groups either in the ITT or in the PP analysis. The Panel notes that this study powered to detect small (3 mm Hg) differences in SBP does not show an effect of IPP and VPP on office SBP or DBP.

In a double-blind, placebo-controlled, parallel trial (Engberink et al., 2008), 135 subjects with elevated SBP were randomly assigned to one of the following four groups: yoghurt drinks devoid of IPP and VPP (placebo) or yoghurt drinks providing 9.2-11.2 mg/day of IPP and VPP obtained from either fermented milk, enzymatic hydrolysis, or chemical synthesis. The intervention lasted eight weeks. A total of 134 subjects completed the intervention. Office and home BP were measured at baseline and at weeks four and eight of the study. Twenty-four hour ambulatory BP measurements were obtained in a sub-group of 58 subjects at the same time points. ANOVA was used on an ITT basis to assess the effect of the intervention on BP. The Panel notes that repeated measures were not appropriately taken into account by ANOVA. Data analysis was repeated after exclusion of non-compliant subjects who consumed <80 % of the test products. Consumption of IPP and VPP did not affect SBP or DBP compared with placebo regardless of the method by which IPP and VPP were obtained, of the methods used to measure BP, and of the level of compliance. The Panel considers that this study does not show an effect of IPP and VPP on office, home, or 24 h ambulatory SBP or DBP.

In a randomised, double-blind, parallel-group, dose-response intervention (De Leeuw et al., 2009), 185 hypertensive subjects received either a placebo or one of three yoghurt drinks providing IPP and VPP at a daily dose of 2.3, 4.6 or 9 mg for eight weeks, after a run-in period of two weeks and followed by a post-intervention follow-up period of two weeks. The primary outcome of the study was changes in SBP. A sample size of 40 per group was calculated to detect a change in SBP of 5 mm Hg with a power of 80 % and  $\alpha=0.05$  (one sided). Office and home BP were measured every two weeks, and 24 h BP measures were obtained at baseline and at week eight. One hundred and sixty-six subjects completed the study and entered data analysis. The effects of treatment over time were assessed by



RM-ANOVA. The Panel notes that it is unclear from the publication whether statistical analyses were performed on an ITT or PP basis. Office SBP significantly decreased in all four groups (including placebo) during the study. When the results of office BP measurements over eight weeks were corrected for the placebo response, no significant differences in SBP or DBP were observed between groups. The percentages of subjects in each intervention group who showed a fall in SBP > 3 mm Hg or who attained an SBP < 140 mm Hg were calculated and analysed *post hoc*. The Panel notes that the *post hoc* response rate analysis was not pre-planned, and considers that no conclusions can be drawn from this analysis for the scientific substantiation of the claim. There were no significant differences in home or 24 h ambulatory BP between groups throughout the study. The Panel considers that this study powered to detect differences in SBP of 5 mm Hg does not show an effect of IPP and VPP on office, home or 24 h ambulatory SBP or DBP.

Van Mierlo et al. (2009) reported the results of two multicentre, randomised, placebo-controlled, cross-over studies, each consisting of two four-week intervention periods separated by a four-week wash-out period. The sample size calculation indicated that for each cross-over study, 48 subjects should be sufficient to detect a 3 mm Hg difference in SBP with a power of 90 % and a one-sided  $\alpha=0.05$ . In Study 1, 69 subjects received a yoghurt drink to which casein hydrolysate had been added in order to provide 10.2 mg/day IPP and VPP or the same yoghurt drink without casein hydrolysate (placebo). Sixty-four subjects completed the study. In Study 2, 93 subjects received a yoghurt drink to which casein hydrolysate (in order to provide 4.6 mg/day IPP and VPP) and potassium (350 mg/day) had been added, or the same yoghurt drink without added IPP, VPP or potassium (placebo). Ninety-one subjects completed this study. Subjects had high-normal BP or grade I hypertension and were not pharmacologically treated for hypertension. The order of the intervention was randomly assigned according to daytime ambulatory BP at baseline. BP was assessed before and after each intervention period both at the office and with a 24 h BP ambulatory monitor. ANCOVA was applied on the ITT population to evaluate the effect of the intervention on BP measures. Selection of subjects who entered the PP analysis and all data analyses were performed before unblinding data. No significant differences between periods were observed for mean 24 h SBP or DBP in either study. Office BP decreased over the course of both studies, but differences between intervention and placebo periods were not significant. The Panel considers that these two studies powered to detect small (3 mm Hg) differences in SBP do not show an effect of IPP and VPP on office or 24 h ambulatory SBP or DBP.

Usinger et al. (2010a; 2010b) enrolled 94 hypertensive subjects into a randomised, double-blind, placebo controlled parallel eight-week study. Subjects were randomised into four groups: one group receiving fermented milk providing 3.6 mg/day IPP and VPP (n=32), a second group which received 1.8 mg/day IPP and VPP (n=32), and two placebo groups which received either 150 ml/day (n=15) or 300 ml/day (n=15) acidified milk devoid of IPP and VPP. Twenty-four hour ambulatory BP was measured at baseline, 4 and 8 weeks. Office BP was measured at baseline, 1, 4 and 8 weeks. Power calculation indicated that the study was adequately powered (no figure provided) to detect a 4 mm Hg difference in 24 h ambulatory BP with 30 subjects per group. Four participants dropped out and 24 h ambulatory BP measurements were incomplete in one case. RM-ANOVA was used to assess differences over time and between groups. The placebo groups were pooled for data analysis. Twenty-four hour ambulatory SBP and DBP declined over time, but no significant difference was found between groups. Similarly, there were no significant differences in office SBP or DBP between groups. The Panel considers that this study powered to detect differences of 4 mm Hg in 24 h ambulatory BP does not show an effect of IPP and VPP on SBP or DBP.

The Panel notes that 15 RCTs (Aihara et al., 2005; Cicero et al., 2010; De Leeuw et al., 2009; Engberink et al., 2008; Itakura et al., 2001; Mizushima et al., 2004; Seppo et al., 2003; Tuomilehto et al., 2004; Turpeinen et al., 2009; Usinger et al., 2010a; Van der Zander et al., 2008a; Van der Zander et al., 2008b; Van Mierlo et al., 2009 two studies; Yoshizawa et al., 2010), of which seven were designed to detect small (3-9 mm Hg) differences in SBP (Aihara et al., 2005; De Leeuw et al., 2009; Usinger et al., 2010a; Van der Zander et al., 2008a; Van der Zander et al., 2008b; Van Mierlo et al., 2009 two studies), did not observe an effect of IPP and VPP on SBP or DBP.

The Panel notes that ten RCTs, nine of which had methodological weaknesses which limit their interpretation, reported an effect of IPP and VPP on office SBP, and that four of these studies also reported an effect of IPP and VPP on office DBP. However, the Panel also notes that 15 RCTs, of which seven were designed to detect small (3-9 mm Hg) differences in SBP, did not observe an effect of IPP and VPP on SBP or DBP.

With respect to possible mechanisms by which IPP and VPP could exert an effect on blood pressure, it is suggested in the information provided that IPP and VPP have the potential to inhibit the angiotensin converting enzyme (ACE), that they could induce the production of nitric oxide, and that they may have an effect on the sympathetic nervous system.

In seven of the human intervention studies (one reported in two publications) described above (Aihara et al., 2005; Cicero et al., 2010; De Leeuw et al., 2009; Engberink et al., 2008; Mizushima et al., 2004; Usinger et al., 2010a; 2010b; Van der Zander et al., 2008b) the effects of IPP and VPP on the activity of the renin-angiotensin-aldosterone system II were assessed by measuring ACE activity or changes in plasma concentrations of renin, aldosterone, angiotensin I or angiotensin. None of these studies showed a statistically significant effect between groups of IPP and VPP on parameters measured which could indicate an inhibition of ACE *in vivo* in humans. In one of the studies (reported in two publications) (Usinger et al., 2010a; 2010b) also plasma noradrenalin concentrations in response to a tilt table test were measured to assess sympathetic activity, and were not statistically significantly different between groups at the end of the study.

One short account of an experiment (Nakamura et al., 1996) carried out for 16 weeks in spontaneously hypertensive rats was provided, which reported that the increase in BP was blunted by the long term consumption of sour milk containing IPP and VPP (doses not indicated). The activity of ACE in the aorta was mentioned to be significantly lower in the intervention group as compared to the control group, while the activity of ACE isolated from other tissues did not differ significantly (data not shown).

The one *in vitro* study (Nakamura et al., 1995) provided, showed that some of the peptides, namely IPP and VPP, produced during milk fermentation by *L. helveticus* and *S. cerevisiae* have an ACE inhibitory activity *in vitro*. The Panel considers that the animal and *in vitro* studies do not provide any additional information on the effect of IPP and VPP on BP in humans.

Additional *ex vivo* and *in vitro* studies related to a potential inhibition of ACE by IPP and VPP were considered in an earlier opinion of the Panel related to a health claim pursuant to Article 13.5 of Regulation (EC) No 1924/2006 (EFSA Panel on Dietetic Products Nutrition and Allergies (NDA), 2011), and did not provide any convincing evidence in support of a proposed mechanism by which IPP and VPP could exert the claimed effect in humans at the proposed dose.

The Panel considers that no convincing data on a possible mechanism by which IPP and VPP could exert an effect on blood pressure in humans at the proposed dose levels have been provided.

In weighing the evidence, the Panel took into account that 15 of the human intervention studies provided, of which seven were adequately powered to detect small between-group differences in SBP, did not observe an effect of IPP and VPP on SBP or DBP; that interpretation of the results from nine out of the ten studies which reported an effect of IPP and VPP on office SBP was limited by methodological weaknesses; that the animal and *in vitro/ex vivo* studies did not provide additional information on the effect of IPP and VPP on BP in humans; and that there is no convincing evidence for a mechanism by which IPP and VPP could exert the claimed effect in humans at the proposed dose.

The Panel concludes that a cause and effect relationship has not been established between the consumption of IPP and VPP and maintenance of normal blood pressure.

## CONCLUSIONS

On the basis of the data presented, the Panel concludes that:

- The food constituent, the tripeptides isoleucine-proline-proline (IPP) and valine-proline-proline (VPP), which is the subject of the health claim, is sufficiently characterised.
- The claimed effect, which is eligible for further assessment, is maintenance of normal blood pressure. The proposed target population is the general population. Maintenance of normal blood pressure is a beneficial physiological effect.
- A cause and effect relationship has not been established between the consumption of isoleucine-proline-proline (IPP) and valine-proline-proline (VPP) and maintenance of normal blood pressure.

## DOCUMENTATION PROVIDED TO EFSA

Health claims pursuant to Article 13 of Regulation (EC) No 1924/2006 for further assessment (No: EFSA-Q-2012-00126, EFSA-Q-2012-00168, EFSA-Q-2012-00169, EFSA-Q-2012-00171). The scientific substantiation is based on the information provided by the Member States in the consolidated list of Article 13 health claims, references that EFSA has received from Member States or directly from stakeholders and the additional information provided by the competent Authority of Germany for further assessment of this claim (available at: <http://www.efsa.europa.eu/en/topics/topic/article13.htm>).

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## APPENDICES

### APPENDIX A

#### BACKGROUND AND TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

The Regulation 1924/2006 on nutrition and health claims made on foods<sup>5</sup> (hereinafter "the Regulation") entered into force on 19<sup>th</sup> January 2007.

Article 13 of the Regulation foresees that the Commission shall adopt a Community list of permitted health claims other than those referring to the reduction of disease risk and to children's development and health. This Community list shall be adopted through the Regulatory Committee procedure and following consultation of the European Food Safety Authority (EFSA).

Health claims are defined as "any claim that states, suggests or implies that a relationship exists between a food category, a food or one of its constituents and health".

In accordance with Article 13 (1) health claims other than those referring to the reduction of disease risk and to children's development and health are health claims describing or referring to:

- a) the role of a nutrient or other substance in growth, development and the functions of the body; or
- b) psychological and behavioural functions; or
- c) without prejudice to Directive 96/8/EC, slimming or weight-control or a reduction in the sense of hunger or an increase in the sense of satiety or to the reduction of the available energy from the diet.

To be included in the Community list of permitted health claims, the claims shall be:

- (i) based on generally accepted scientific evidence; and
- (ii) well understood by the average consumer.

Member States provided the Commission with lists of claims as referred to in Article 13 (1) by 31 January 2008 accompanied by the conditions applying to them and by references to the relevant scientific justification. These lists have been consolidated into the list which forms the basis for the EFSA consultation in accordance with Article 13 (3).

#### ISSUES THAT NEED TO BE CONSIDERED

##### IMPORTANCE AND PERTINENCE OF THE FOOD<sup>6</sup>

Foods are commonly involved in many different functions<sup>7</sup> of the body, and for one single food many health claims may therefore be scientifically true. Therefore, the relative importance of food e.g. nutrients in relation to other nutrients for the expressed beneficial effect should be considered: for functions affected by a large number of dietary factors it should be considered whether a reference to a single food is scientifically pertinent.

It should also be considered if the information on the characteristics of the food contains aspects pertinent to the beneficial effect.

<sup>5</sup> OJ L12, 18/01/2007

<sup>6</sup> The term 'food' when used in this Terms of Reference refers to a food constituent, the food or the food category.

<sup>7</sup> The term 'function' when used in this Terms of Reference refers to health claims in Article 13(1)(a), (b) and (c).

## SUBSTANTIATION OF CLAIMS BY GENERALLY ACCEPTABLE SCIENTIFIC EVIDENCE

Scientific substantiation is the main aspect to be taken into account to authorise health claims. Claims should be scientifically substantiated by taking into account the totality of the available scientific data, and by weighing the evidence, and shall demonstrate the extent to which:

- (a) the claimed effect of the food is beneficial for human health,
- (b) a cause and effect relationship is established between consumption of the food and the claimed effect in humans (such as: the strength, consistency, specificity, dose-response, and biological plausibility of the relationship),
- (c) the quantity of the food and pattern of consumption required to obtain the claimed effect could reasonably be achieved as part of a balanced diet,
- (d) the specific study group(s) in which the evidence was obtained is representative of the target population for which the claim is intended.

EFSA has mentioned in its scientific and technical guidance for the preparation and presentation of the application for authorisation of health claims consistent criteria for the potential sources of scientific data. Such sources may not be available for all health claims. Nevertheless it will be relevant and important that EFSA comments on the availability and quality of such data in order to allow the regulator to judge and make a risk management decision about the acceptability of health claims included in the submitted list.

The scientific evidence about the role of a food on a nutritional or physiological function is not enough to justify the claim. The beneficial effect of the dietary intake has also to be demonstrated. Moreover, the beneficial effect should be significant i.e. satisfactorily demonstrate to beneficially affect identified functions in the body in a way which is relevant to health. Although an appreciation of the beneficial effect in relation to the nutritional status of the European population may be of interest, the presence or absence of the actual need for a nutrient or other substance with nutritional or physiological effect for that population should not, however, condition such considerations.

Different types of effects can be claimed. Claims referring to the maintenance of a function may be distinct from claims referring to the improvement of a function. EFSA may wish to comment whether such different claims comply with the criteria laid down in the Regulation.

## WORDING OF HEALTH CLAIMS

Scientific substantiation of health claims is the main aspect on which EFSA's opinion is requested. However, the wording of health claims should also be commented by EFSA in its opinion.

There is potentially a plethora of expressions that may be used to convey the relationship between the food and the function. This may be due to commercial practices, consumer perception and linguistic or cultural differences across the EU. Nevertheless, the wording used to make health claims should be truthful, clear, reliable and useful to the consumer in choosing a healthy diet.

In addition to fulfilling the general principles and conditions of the Regulation laid down in Article 3 and 5, Article 13(1)(a) stipulates that health claims shall describe or refer to "the role of a nutrient or other substance in growth, development and the functions of the body". Therefore, the requirement to describe or refer to the 'role' of a nutrient or substance in growth, development and the functions of the body should be carefully considered.

The specificity of the wording is very important. Health claims such as "Substance X supports the function of the joints" may not sufficiently do so, whereas a claim such as "Substance X helps maintain the flexibility of the joints" would. In the first example of a claim it is unclear which of the various functions of the joints is described or referred to contrary to the latter example which specifies this by using the word "flexibility".

The clarity of the wording is very important. The guiding principle should be that the description or reference to the role of the nutrient or other substance shall be clear and unambiguous and therefore be specified to the extent possible i.e. descriptive words/ terms which can have multiple meanings should be avoided. To this end, wordings like "strengthens your natural defences" or "contain antioxidants" should be considered as well as "may" or "might" as opposed to words like "contributes", "aids" or "helps".

In addition, for functions affected by a large number of dietary factors it should be considered whether wordings such as "indispensable", "necessary", "essential" and "important" reflects the strength of the scientific evidence.

Similar alternative wordings as mentioned above are used for claims relating to different relationships between the various foods and health. It is not the intention of the regulator to adopt a detailed and rigid list of claims where all possible wordings for the different claims are approved. Therefore, it is not required that EFSA comments on each individual wording for each claim unless the wording is strictly pertinent to a specific claim. It would be appreciated though that EFSA may consider and comment generally on such elements relating to wording to ensure the compliance with the criteria laid down in the Regulation.

In doing so the explanation provided for in recital 16 of the Regulation on the notion of the average consumer should be recalled. In addition, such assessment should take into account the particular perspective and/or knowledge in the target group of the claim, if such is indicated or implied.

## **TERMS OF REFERENCE**

### **HEALTH CLAIMS OTHER THAN THOSE REFERRING TO THE REDUCTION OF DISEASE RISK AND TO CHILDREN'S DEVELOPMENT AND HEALTH**

EFSA should in particular consider, and provide advice on the following aspects:

- Whether adequate information is provided on the characteristics of the food pertinent to the beneficial effect.
- Whether the beneficial effect of the food on the function is substantiated by generally accepted scientific evidence by taking into account the totality of the available scientific data, and by weighing the evidence. In this context EFSA is invited to comment on the nature and quality of the totality of the evidence provided according to consistent criteria.
- The specific importance of the food for the claimed effect. For functions affected by a large number of dietary factors whether a reference to a single food is scientifically pertinent.

In addition, EFSA should consider the claimed effect on the function, and provide advice on the extent to which:

- the claimed effect of the food in the identified function is beneficial.
- a cause and effect relationship has been established between consumption of the food and the claimed effect in humans and whether the magnitude of the effect is related to the quantity consumed.
- where appropriate, the effect on the function is significant in relation to the quantity of the food proposed to be consumed and if this quantity could reasonably be consumed as part of a balanced diet.
- the specific study group(s) in which the evidence was obtained is representative of the target population for which the claim is intended.

- the wordings used to express the claimed effect reflect the scientific evidence and complies with the criteria laid down in the Regulation.

When considering these elements EFSA should also provide advice, when appropriate:

- on the appropriate application of Article 10 (2) (c) and (d) in the Regulation, which provides for additional labelling requirements addressed to persons who should avoid using the food; and/or warnings for products that are likely to present a health risk if consumed to excess.

## **APPENDIX B**

### **EFSA DISCLAIMER**

The present opinion does not constitute, and cannot be construed as, an authorisation to the marketing of the food/food constituent, a positive assessment of its safety, nor a decision on whether the food/food constituent is, or is not, classified as foodstuffs. It should be noted that such an assessment is not foreseen in the framework of Regulation (EC) No 1924/2006.

It should also be highlighted that the scope, the proposed wordings of the claims and the conditions of use as proposed in the Consolidated List may be subject to changes, pending the outcome of the authorisation procedure foreseen in Article 13(3) of Regulation (EC) No 1924/2006.

**GLOSSARY AND ABBREVIATIONS**

ACE	Angiotensin converting enzyme
ANCOVA	Analysis of covariance
ANOVA	Analysis of variance
AUC	Area under the curve
BP	Blood pressure
DBP	Diastolic blood pressure
IPP	Isoleucyl-prolyl-proline
ITT	Intention-to-treat
PP	Per protocol
RCT	Randomised controlled trial
RM	Repeated measures
SBP	Systolic blood pressure
VPP	Valyl-prolyl-proline